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Comparative Research of Gametophytes of *Olfersia alata* and *Olfersia cervina* (Dryopteridaceae)

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ABSTRACT.—The prothallial development of gametophytes of *Olfersia alata* and *Olfersia cervina* (Dryopteridaceae) is described and compared. Spores are monolete, ellipsoid, and with broadly winged perispore. Germination is *Vittaria*-type and the prothallial development is *Aspidium*-type. Adult gametophytes are cordiform-spatulate to cordiform-reniform, with marginal and superficial trichomes. Gametangia are of the type commonly found on leptosporangiate homosporous ferns. Differences between the two species of genus include size of the spores, width of the perispore, germination time, size of the trichomes, and time of formation of the gametangia. These two species share some features with some species of *Arachniodes*, *Cyrtomium*, *Dryopteris*, *Phanerophlebia*, and *Polystichum*, such as type of germination and prothallial development and trichomes. They differ from *Didymochlaena truncatula*, which has prothallial development of the *Adiantum*-type and lacks trichomes on the sexual phase.

The genus *Olfersia* Raddi (Dryopteridaceae), has two species: *Olfersia alata* C. Sánchez & García Caluff and *Olfersia cervina* (L.) Kunze. *Olfersia alata* is endemic to Cuba; its main characteristics are all sterile pinnae have decurrent bases, and fertile leaves which are smaller and have fewer pinna pairs than the vegetative leaves. It grows in mountainous mesophytic forests, between 350–400 m (Sánchez *et al.*, 1991). *Olfersia cervina* is widely distributed in the tropics, from Southern Mexico (Chiapas, Oaxaca, Veracruz), to Southeastern Brazil and the West Indies. In this species the bases of the sterile pinnae are not decurrent onto the rachis, and pinnae are short-petiolulate. It grows between 450–1000 m in damp tropical forests, on rocky and very shady banks (Moran, 1986, 1995; Riba and Pérez-García, 1999). Both taxa are usually terrestrial, rarely hemiepiphytic, with a short trailing rhizome. Leaves are markedly dimorphic, sori are exindusiate and linear to oblong, and spores are monolete, echinulate with a broad perispore.

This paper complements existing information about the morphogenesis of the gametophytic phase of dryopteriod ferns and, particulary, focuses on gametophytes of *Olfersia*. We hope to contribute in this way to the knowledge of the sexual phase of Mexican ferns.

MATERIALS AND METHODS

Spores of *O. cervina* were collected from living plants from the following site: Lote 69 in "Los Tuxtlas" Biological Station, between Laguna Azul and Laguna Seca, Mun. of San Andrés Tuxtla, in the state of Veracruz, Mexico; vouchers are in UAMIZ (*AMR 202* and *AMR 251*). Spores of *O. alata* were

collected by Carlos Sánchez and L. del Risco (*77825*) near Farallones de Moa, Farallón Redondo and La Escondida, Mun. Moa, Holguín province, Cuba; the voucher is in HAJM.

Fertile pinnae were kept in paper bags until spores were shed. Subsequently, the spores and shed material were passed through a sieve (with pores 0.074 mm in diameter) in order to eliminate traces of sporangia and indusia. Spores of each species were sown at an average density of 150–200 spores per cm^2 in two small pots with a mixture of black soil and organic matter and in 30 Petri dishes, 5 cm in diameter, containing Thompson's solution of mineral salts and agar on a sterile nutrient medium (Klekowski, 1969).

Petri dishes and pots were kept inside transparent plastic bags in order to avoid contamination and desiccation, with a photoperiod of 12 h light/darkness, with artificial light (75 Watt lamps, daylight) and a temperature of 23–25° C (Mendoza *et al.*, 1999a, 1999b). Two dishes were kept in darkness in order to determine photoblastism. After 100 days, none of the spores grown in darkness had germinated.

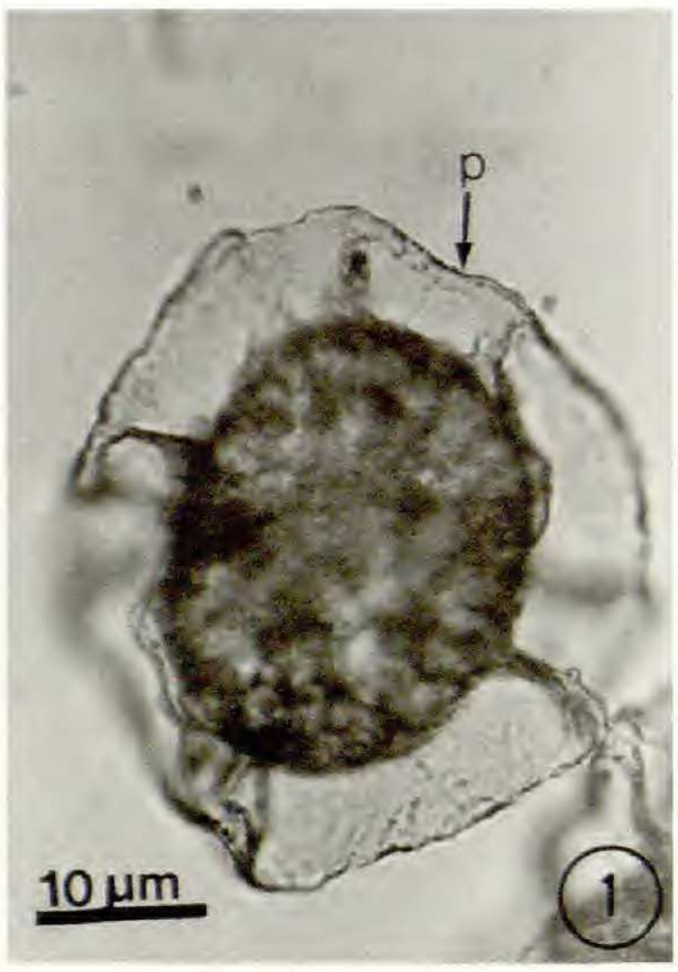
All pictures of microscopic material were taken from living material grown in the laboratory.

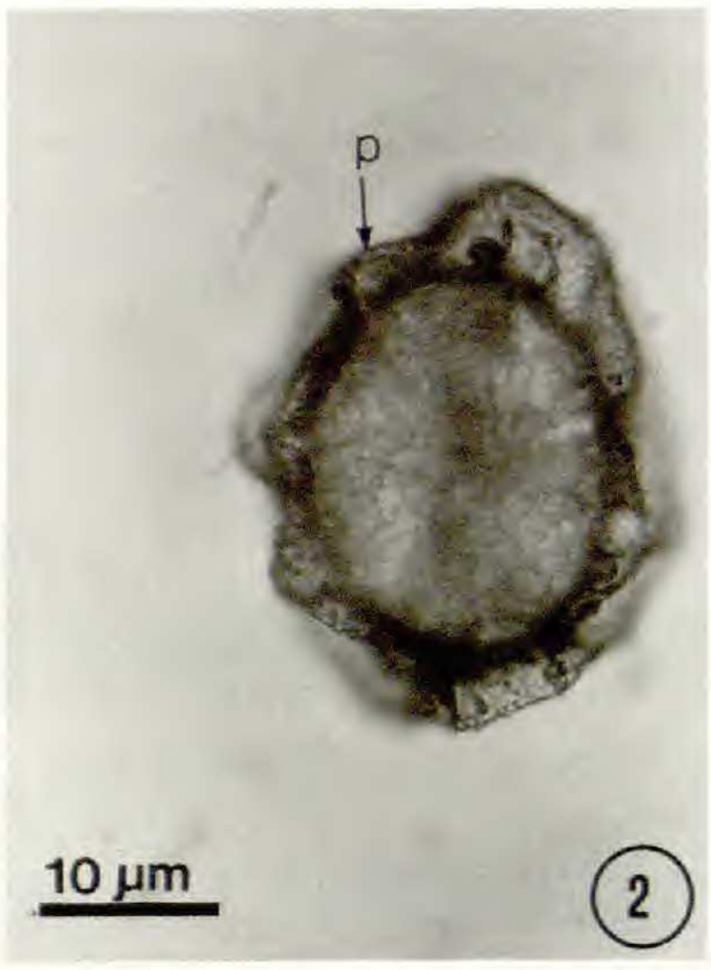
Results

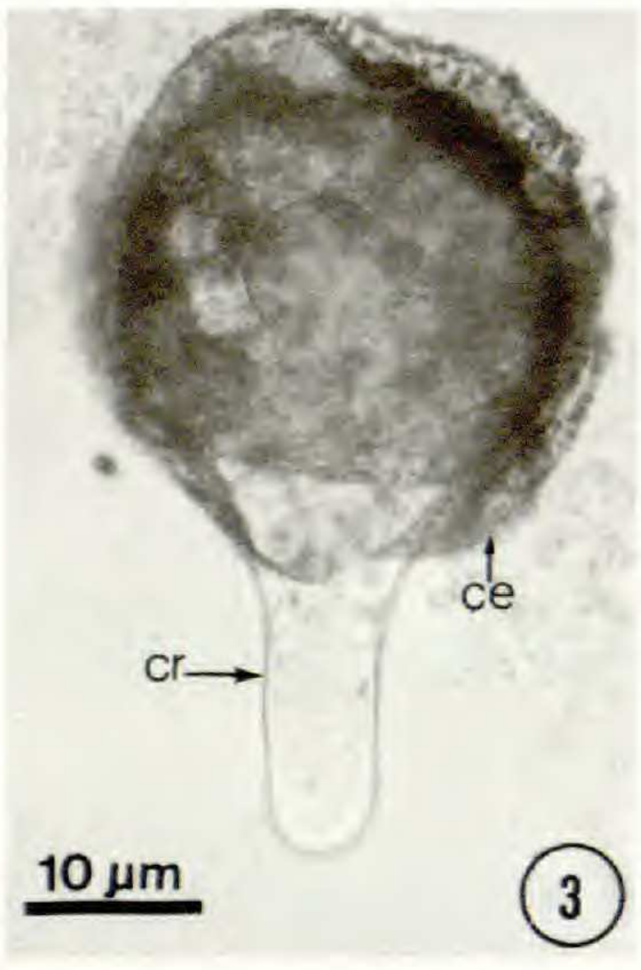
Spores of both species are monolete, nearly spherical, with a light brown perispore. Spores of *Olfersia alata* measure (64) 73 (83) × (49) 53 (55) μm, including the winged perine around the spore; the perispore measures (15) 16 (20) μm wide (Fig. 1). Spores of *O. cervina* measure (44) 48 (51) × (37) 39 (40) μm, also including the winged perine which measures (5) 6 (8) μm wide (Fig. 2). Spores of *O. alata* are larger than those of *O. cervina* primarily due to the size of the perispore. These measurements were obtained from an average sample of fifty spores per species.

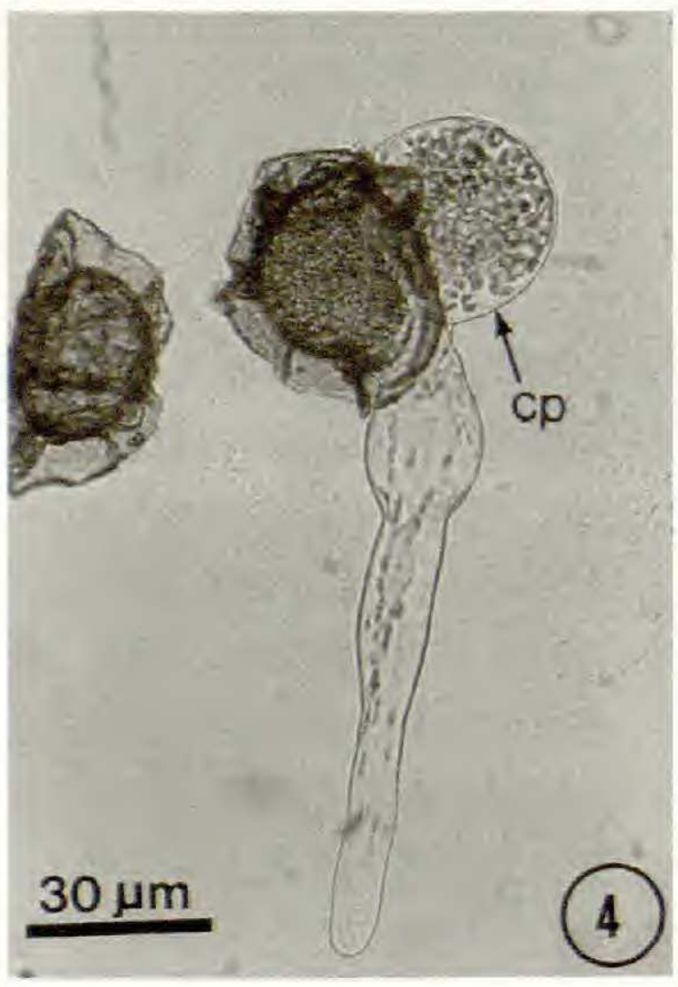
Germination is *Vittaria*-type (Nayar & Kaur, 1971) in both species. In *Olfersia alata* germination began 20–23 days after spores were sown, whereas in *O. cervina* it began 8–12 days after sowing. Gametophytes of both species first develop a rhizoid, which is short, hyaline, and without chloroplasts. The first prothallial cell is short and oval; division begins in this cell with a transverse wall and ultimately forms a short germ-filament, 2–4 cells long. This filament eventually ends in an apical trichome. During this stage of development, the spores retain their coat (Figs. 3–5).

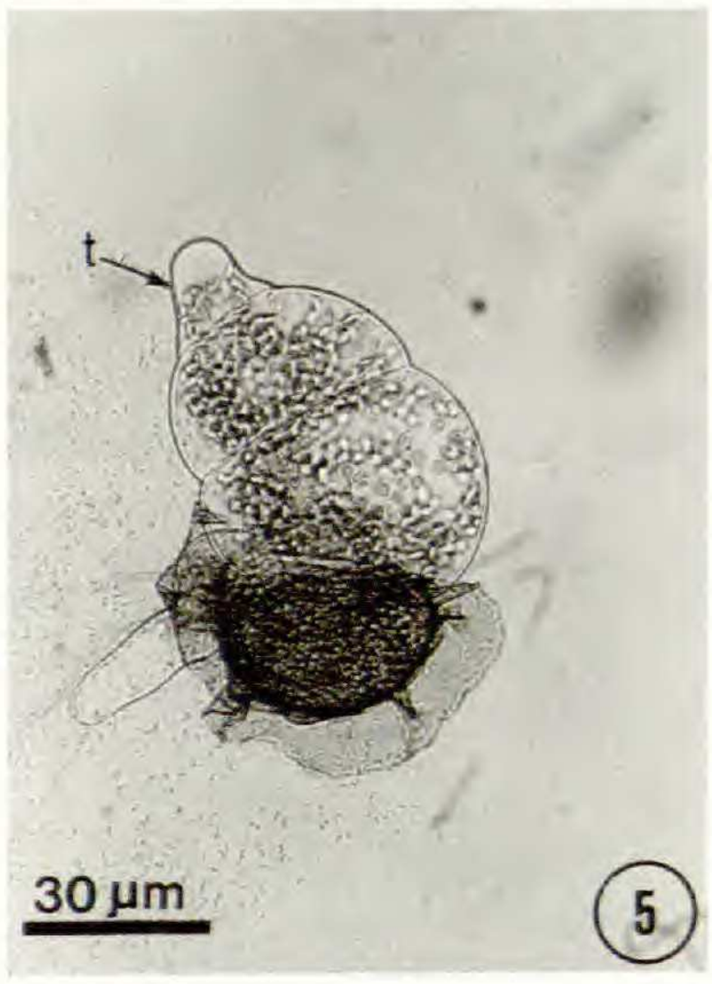
In *O. alata*, the prothallial plate begins to develop approximately 25 days after spore germination from intercalary cells of the filaments which undergo longitudinal divisions (Figs. 6–7). In some cases, the terminal cell of the filament, after producing a trichome, will divide longitudinally in such a manner that the trichome is placed over one of the daughter cells, which will remain inactive until other cells develops into a gametophytic plate. This plate, from which a meristematic cell will emerge, is usually asymmetric

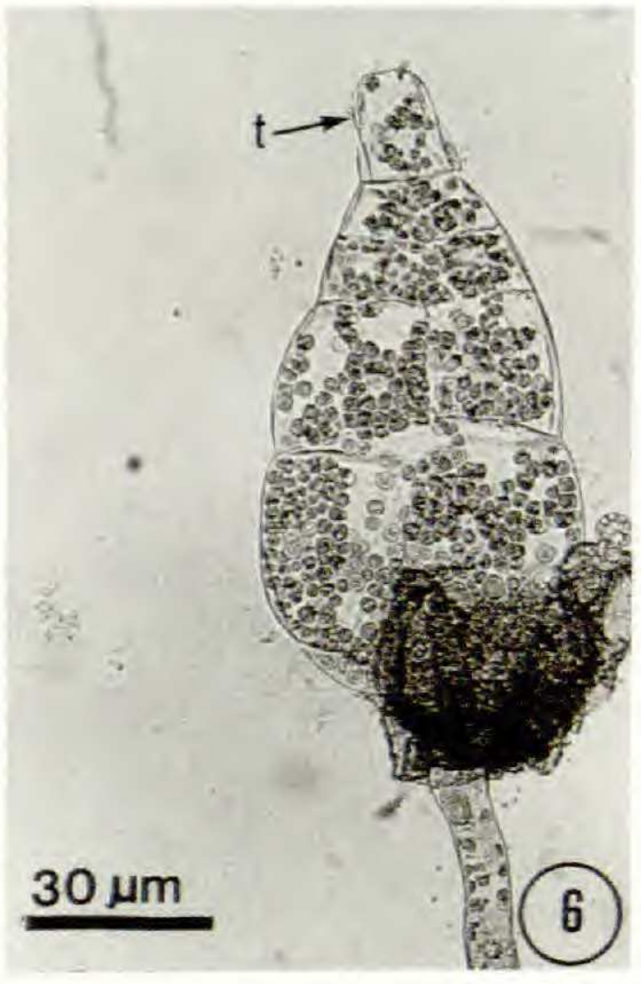


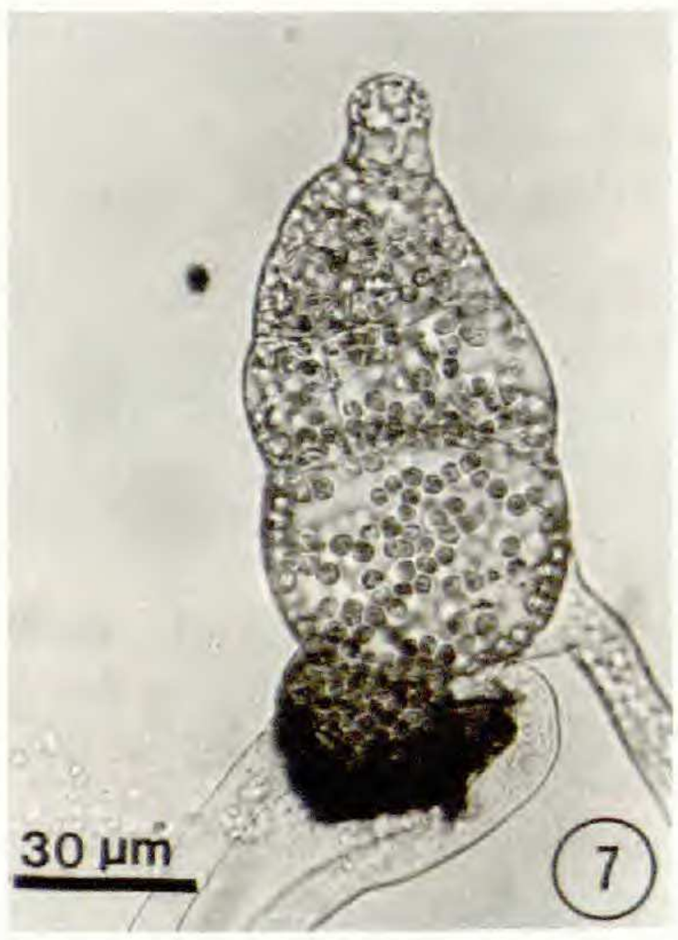


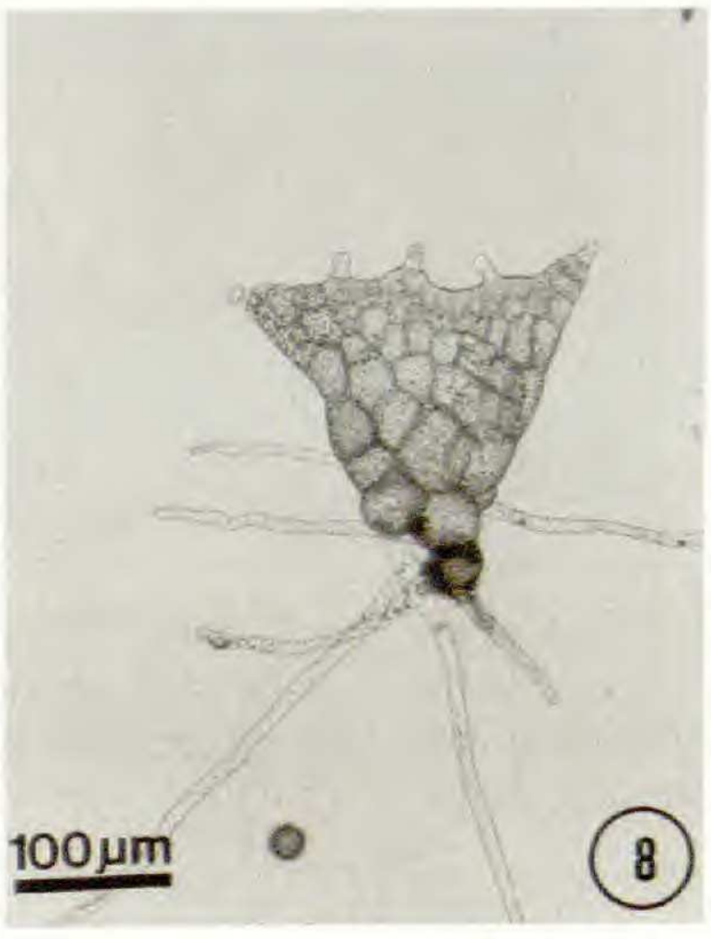


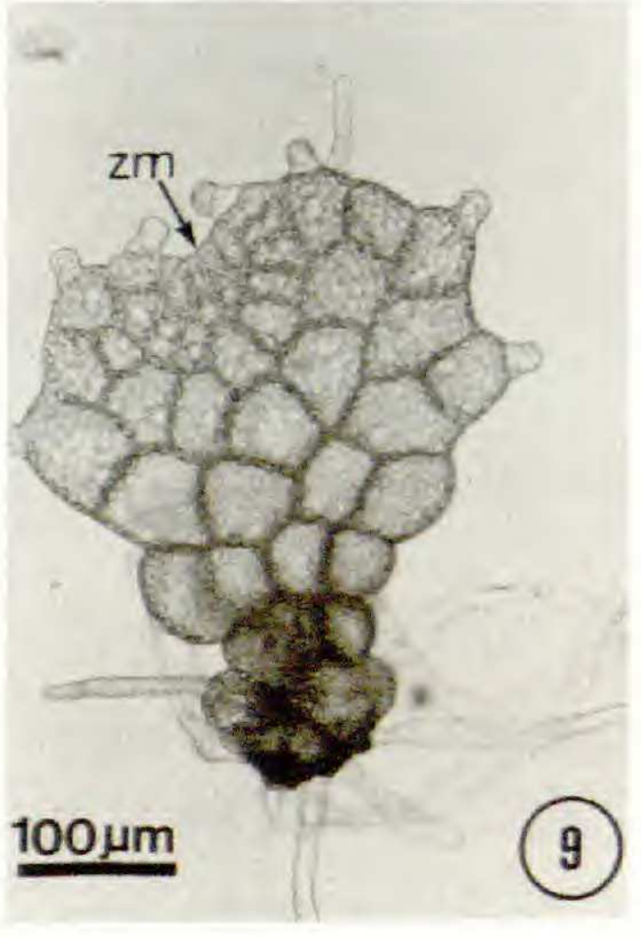




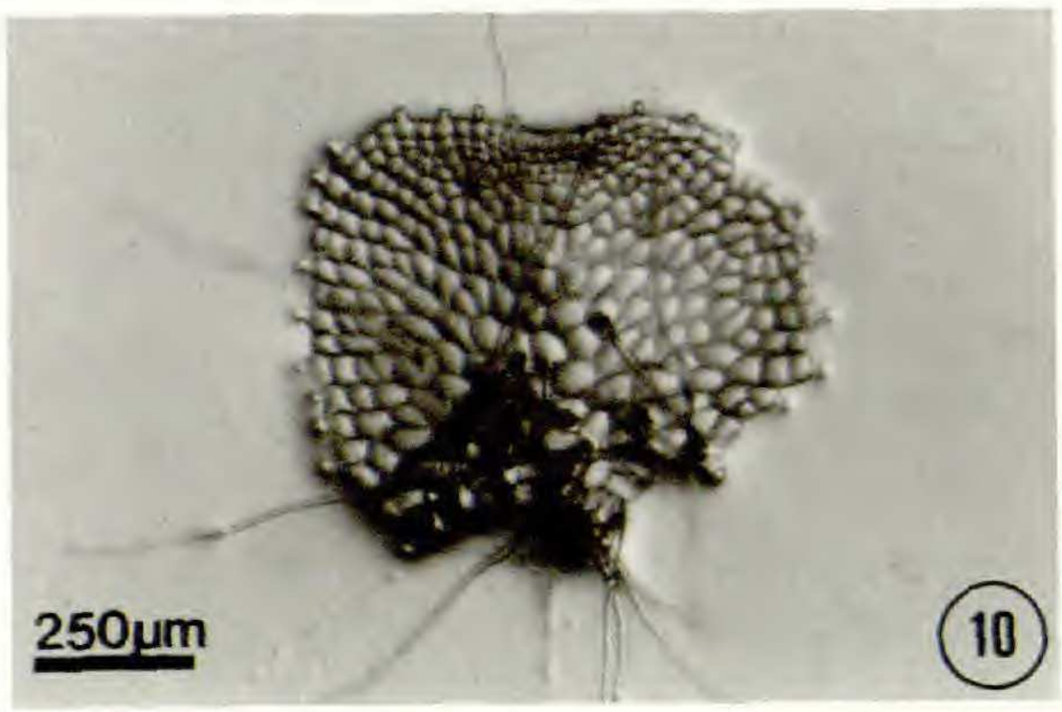


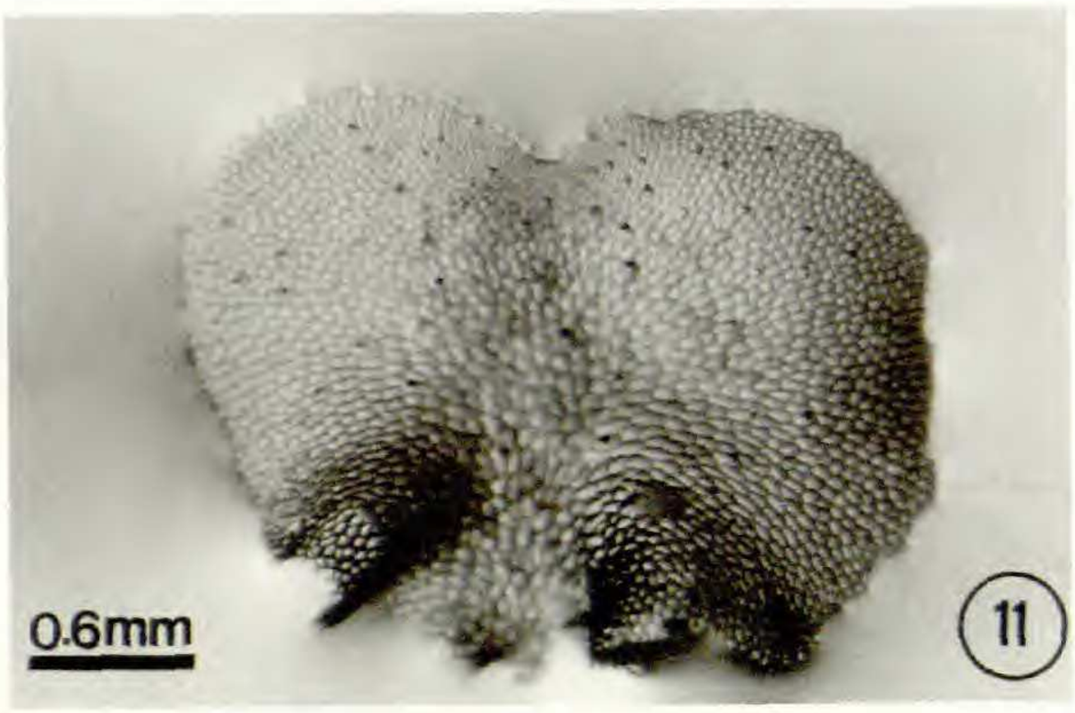


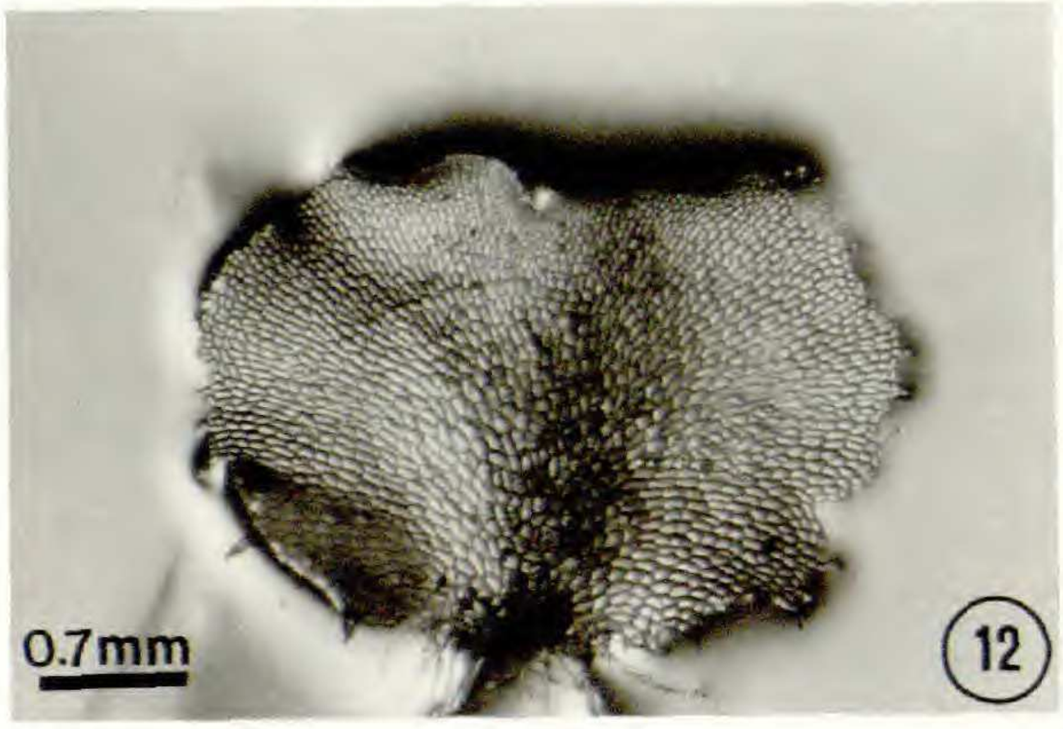


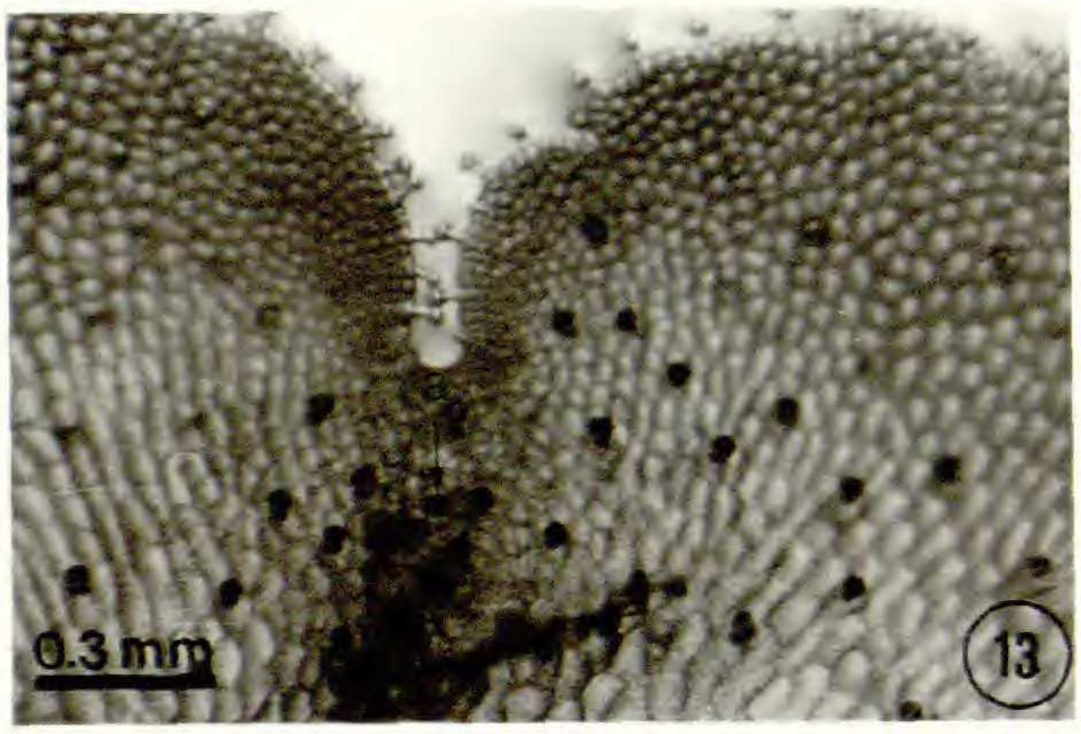


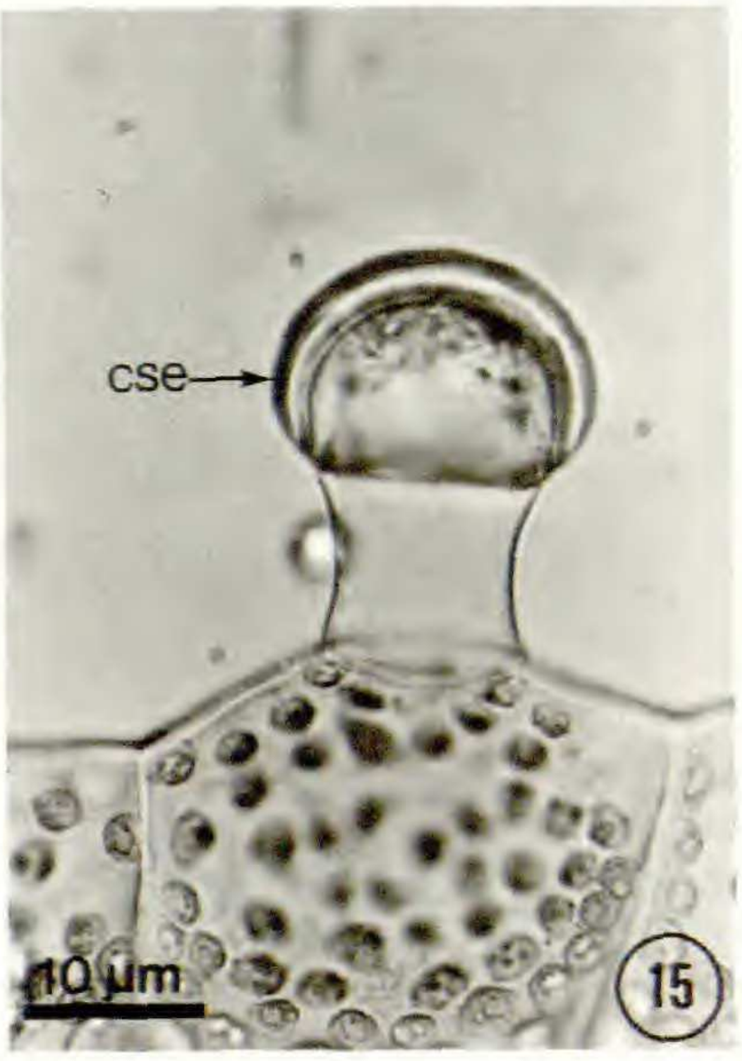
FIGS. 1–9. Spores, germination, and filamentous and laminar phases of *Olfersia*. 1. Spore, *O. alata*. 2. Spore, *O. cervina*. 3–4. Initial stages of germination. 3. *O. cervina* (8 days). 4. *O. alata* (22 days). 5–7. Germ filament. 5–6. *O. alata* (22 days). 7. *O. cervina* (22 days). 8–9. Young laminar phases. 8. *O. alata* (38 days). 9. *O. cervina* (35 days). ce = cover of spores, cp = prothallial cell, cr = rhizoid cell, p = perispore, t = trichome, zm = meristematic zone.

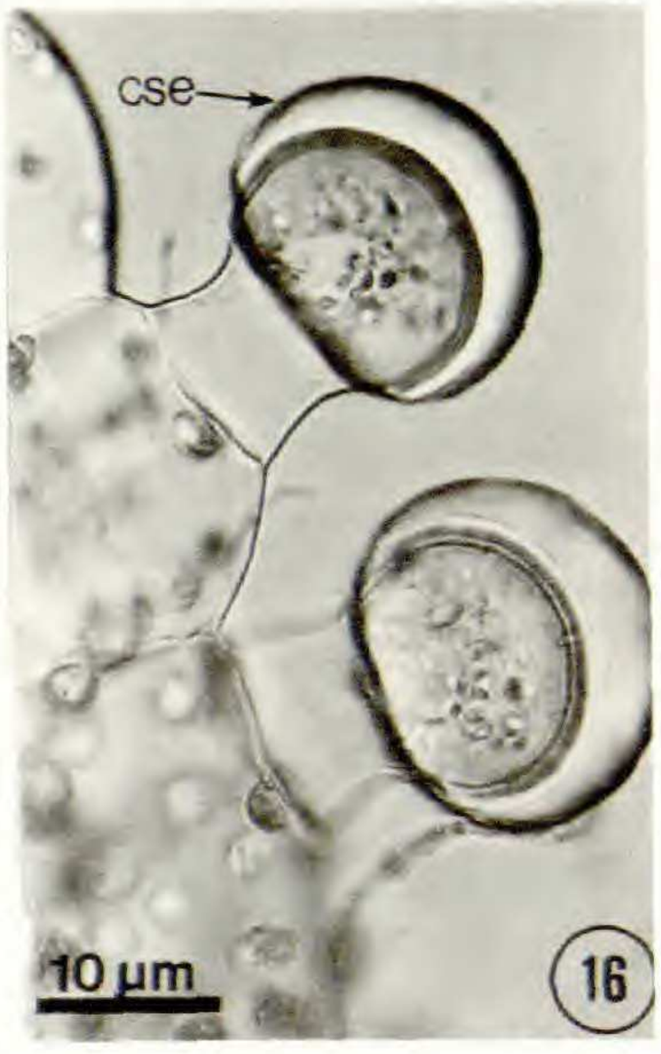






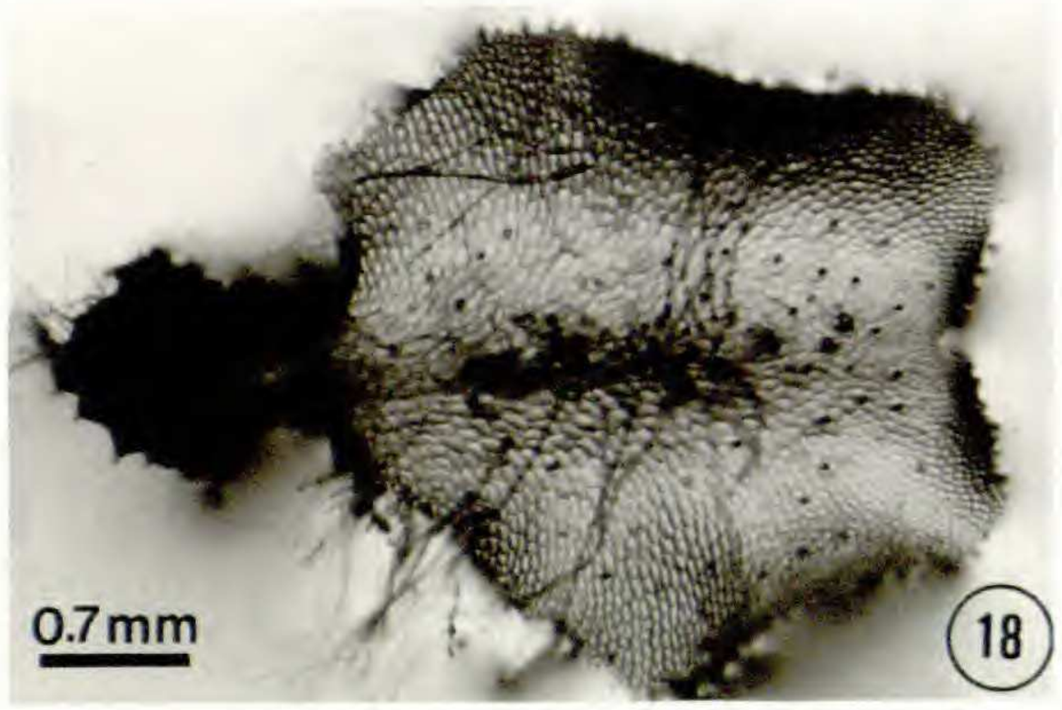


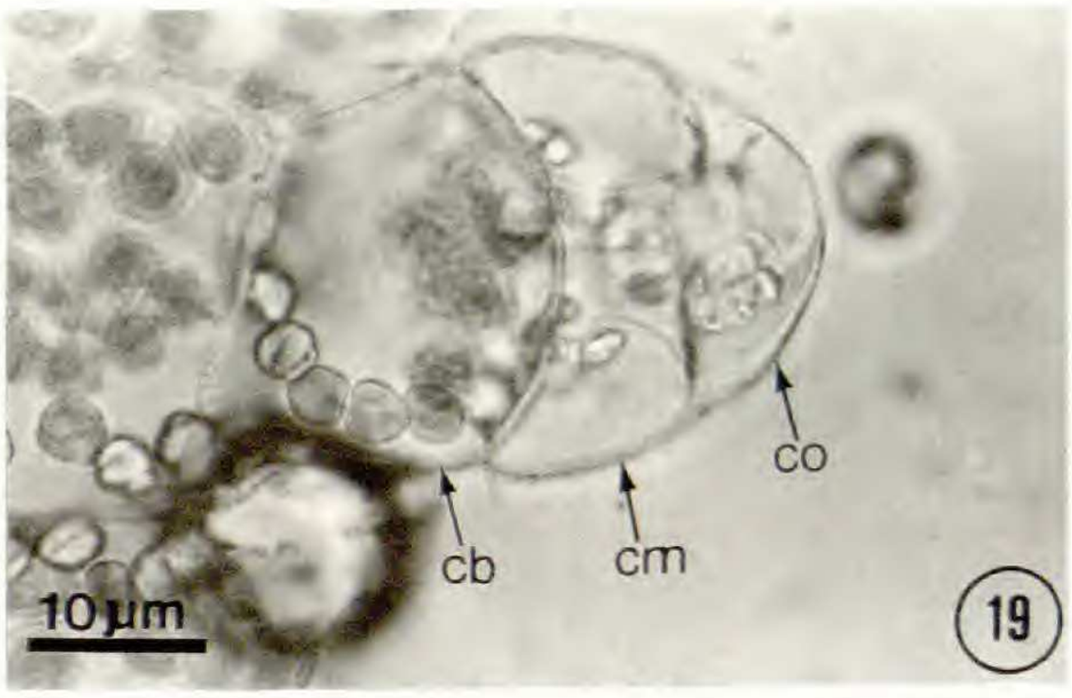


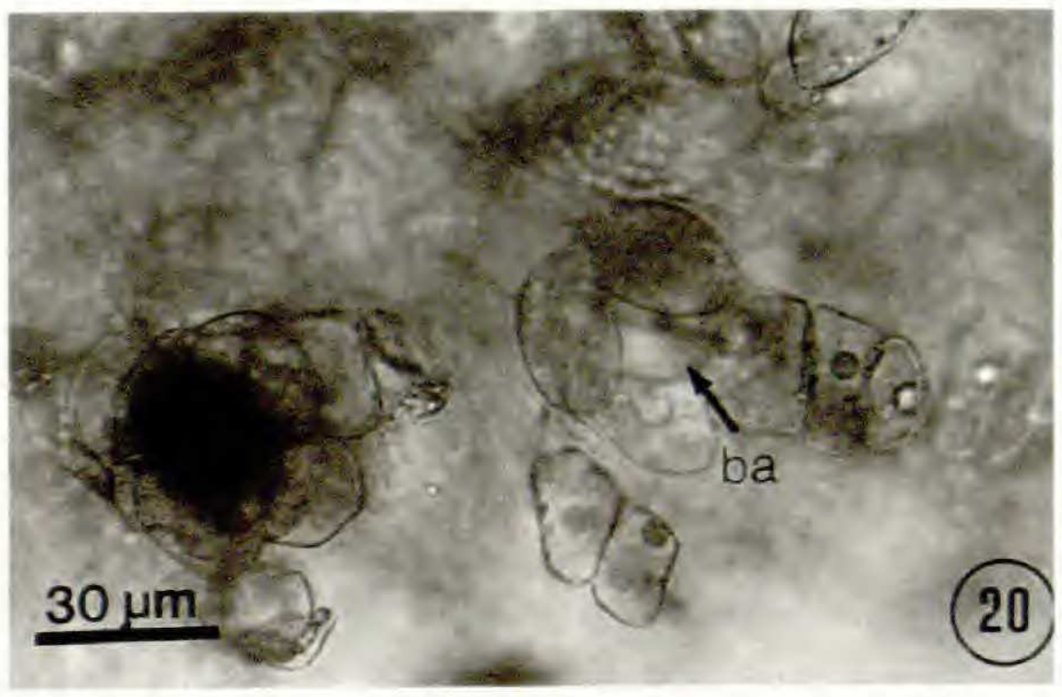


FIGS. 10–16. Laminar gametophytes and secretory, unicellular, capitate trichomes of *Olfersia*. 10–11. *O. cervina* (41 and 100 days). 12–13. *O. alata* (95 and 138 days). 14–16. Trichomes. 14. *O. cervina* (175 days). 15. *O. alata* (149 days). 16. *O. cervina* (175 days). a = archegonia, cse = extracellular secretion cover, t = trichome.

with an apex that continues to change and form a notch. Finally, after 90–100 days, the prothallial plate becomes cordate, the so-called Aspidium-type prothallial plate development (Nayar & Kaur, 1969; Figs. 8–10). Afterwards, a cushion bearing the gametangia forms and the adult gametophyte is cordiform-spatulate with many marginal and superficial trichomes. The formation







FIGS. 17–20. Adult gametophytes and gametangia of *Olfersia*. 17–18. Adult phases of *O. alata* (109–158 days). 19–20. Gametangia. 19. Antheridium, *O. cervina* (246 days). 20. Mouths of archegonium, *O. alata* (138 days). ba = mouth of archegonium, cb = basal cell, cm = ring cell, co = opercular cell.

of the prothallial plate in *O. cervina* takes less time, beginning on day 15. The pattern of prothallial development is the same as in *O. alata*. The first adult cordiform gametophytes are completely differentiated 60–80 days after the spores were sown (Figs. 12–13).

Trichomes are unicellular, capitate, and secretory (Fig. 14). In *O. alata* they measure approximately 36 µm long by 23 µm wide at the base. The apical third of the trichome is globose, 17 µm high by 24 µm wide, with a thin cover of extracellular secretion ca. 3 µm thick (Fig. 15). *Olfersia cervina* trichomes are 34 µm long by 20 µm wide at the base and the apical third of the trichome is globose, 21 µm high by 26 µm wide, with an extracellular secretion 8 µm thick (Fig. 16). These measurements are from mature trichomes, found at the middle basal region of the gametophytes.

The gametangia are typical of leptosporangiate homosporous ferns. They begin differentiating between days 120–244 in *O. cervina* while in *O. alata* they develop between days 100–150. Antheridia are distributed on the lower surface of the plate on the basal half of the cushion (Fig. 19). Antheridia are globose and consist of a basal cell, a ring cell, and an opercular cell. These three cells surround the androgenous cell.

In both species, the necks of the archegonia, have four tiers of neck cells. Archegonia are found on the central region of the plate, on the cushion, and

TABLE 1. Comparison of different stages of the prothallial development of *Olfersia alata*, and *O. cervina* with other genera and species of Dryopteridaceae.

	Spores	Type of germination	Filamentous phase	Type of prothallial development and adult form	Trichomes	Antheridium	Archegonium
[1,2]*Arachniodes*	Monolete, with perispore, measuring 30 42 μm	*Vittaria*	Long filaments (2-5 cells), with an apical trichome	*Aspidium*, cordiform gametophytes with lacerate margins	Unicellular, capitate, with a thin coat of extracellular secretion 2 :m	3 cells	4 rows of cells, each row with 4–6 cells
[1]*Cyrtomium*	Monolete with perispore, measuring 32 45 μm	*Vittaria*	Long filaments (2–5 cells), with an apical trichome	*Aspidium*, cordiform gametophytes, with lacerate margins	Unicellular, capitate	3 cells	4 rows of cells
[2]*Didymochlaena*	Monolete with perispore, measuring 30 37 μm	*Vittaria*	Short filaments (2–3 cells), apical trichome absent	*Adiantum*, cordiform-reniform gametophytes with entire margins	Absent throughout development	3–4 cells	4 rows of cells, each row with 4–6 cells
[3]*Dryopteris*	Monolete with perispore, measuring 36 51 μm	*Vittaria*	Long filaments (2–5 cells), with an apical trichome	*Aspidium*, cordiform-reniform gametophytes with lacerate margins	Unicellular, capitate	3 cells	4 rows of cells, each row with 4–5 cells
Olfersia alata	Monolete with a broadly winged perispore, measuring 53 73 μm	*Vittaria*	Short filaments (2–4 cells), with an apical trichome	*Aspidium*, spatulate-cordiform gametophytes with entire margins	Unicellular, capitate with an extra-cellular secretion coat 3 :m thick	3 cells	4 rows of cells, each row with 4–5 cells

TABLE 1. Continued.

	Spores	Type of germination	Filamentous phase	Type of prothallial development and adult form	Trichomes	Antheridium	Archegonium
O. cervina	Monolete with a winged perispore, measuring 39 48 μm	*Vittaria*	Short filaments (2–4 cells), with an apical trichome	*Aspidium*, spatulate-cordiform gametophytes with entire margins	Unicellular, capitate with an extra-cellular secretion coat 8 :m thick	3 cells	4 rows of cells, each row with 4–5 cells
[1]*Polystichum*	Monolete with perispore, measuring 34 45 μm	*Vittaria*	Long filaments (2–8 cells), without trichomes	*Aspidium*, cordiform gametophytes with lacerate margins	Unicellular, pappilate capitate, secretors and non secretors	3–4 cells	4 rows of cells
[4]*Phanerophlebia*	Monolete with perispore, measuring 25 33 μm	*Vittaria*	Long filaments (2–6 cells), with an apical trichome	*Aspidium*, spatulate-cordiform gametophyes with lacerate margins	Unicellular, capitate, with an extra-cellular secretion coat 1.5 :m thick	3–4 cells	4 rows of cells, each row with 4–6 cells

[1] Chandra & Nayar 1970, [2] Mendoza *et al.* 1999a, 1999b,[3] Pérez-García *et al.* 1999. [4] Mendoza, unpublished.

near the meristematic zone. The necks are oriented toward the basal region of the gametophytes (Figs. 13, 20). Two hundred days after sowing the spores, the young sporophytes had not yet formed.

Discussion and Conclusions

There is literature dealing with the morphology of the gametophytic phase of ferns closely related to *Olfersia*, of the Dryopteridaceae (both Old and New World), *e.g.*, *Arachniodes*, *Cyrtomium*, *Didymochlaena*, *Dryopteris*, and *Polystichum* (Atkinson, 1973; Chandra & Nayar, 1970; Cousens, 1975; Kaur, 1977; Mendoza et al., 1999a, 1999b; Pérez-García et al., 1999; Stokey & Atkinson, 1954).

Spores of *O. alata* average 73 × 53 μm, including the winged perispore; spores of *O. cervina* average of 48 × 39 μm. Spores of *O. alata* seem much larger, but in reality, if the perispore is not considered, the spores are 38 × 23 μm, and the winged perine is 16 μm wide or more in its widest part. Spores of *O. cervina* are 39 × 31 μm and the perispore is approximately 6 μm wide at its widest point and tends to be more spherical, which is an indication that the spores of *O. alata* are a little smaller than those of *O. cervina*. (Figs. 1, 2).

Both species share the same germination pattern, *Vittaria*-type, which is the most common type in ferns. In this type, the rhizoid develops first after the formation of a wall perpendicular to the polar axis of the spores. Eventually, the first prothallial cell divides by means of the formation of a perpendicular wall thus giving rise to two cells. The apical cell then divides again, giving rise to a short filament 2–4 cells long. The time for germination differs between these two species; spores of *O. cervina* germinate faster (8–12 days) compared to spores of *O. alata* (20–22 days).

Prothallial development in both species is of the *Aspidium*-type in which the germ filament commonly ends in a trichome, and the prothallial plate is formed by the activity of the intercalary cells of the filament. The adult gametophyte develops faster in *O. cervina* (60–80 days) than in *O. alata* (90–100 days).

Trichomes differ in size and in the thickness of the extracellular secretion; the longest ones, belongins to *O. alata* (36 × 23 μm), have a thinner extracellular secretion (3 μm), whereas trichomes of *O. cervina* are slightly shorter (34 × 20 μm) and have a thicker (8 μm) extracellular secretion.

Olfersia alata and *O. cervina* share features with the following dryopterid genera: *Arachniodes*, *Cyrtomium*, *Dryopteris*, *Phanerophlebia*, and *Polystichum* (Atkinson, 1973; Chandra and Nayar, 1970; Cousens, 1975; Mendoza *et al.*, 1999b; Pérez-García *et al.*, 1999). These genera all have monolete spores with perispore, a *Vittaria*-type germination pattern and an *Aspidium*-type prothallial development. However the two *Olfersia* species differ from the rest in the shape of their trichomes, which are short and wider at the base, capitate, with a globose apex, and with a dense extracellular secretion.

The gametophyte margins are entire in *Olfersia*, compared with the lacerate margins of species of the other genera. These other genera also have longer, capitate trichomes, with very thin extracellular secretions distributed on the lacerate margins and on the surfaces of the plate (Table 1).

Olfersia alata and *O. cervina*, together with the above mentioned taxa, share some features with *Didymochlaena truncatula*, such as the monolete spores and *Vittaria*-type germination. This last species differs from the rest in that is has a prothallial development of the *Adiantum*-type, characterized by a differentiation of an apical meristematic cell during the early stages of the plate's formation. The gametophytes of *Didymochlaena*, are completely glabrous throughout their development, in contrast to the other species of Dryopteridaceae mentioned.

Based on our results, we conclude that *Olfersia alata* and *O. cervina* share characteristics such as the *Vittaria*-type germination pattern, *Aspidium*-type prothallial development, and unicellular capitate trichomes with a uniform extracellular secretion. These same characteristics are characteristic of species of *Arachniodes*, *Cyrtomium*, *Dryopteris*, *Phanerophlebia*, and *Polystichum* (Table 1). The most common feature of all of these genera is the development of an apical trichome during the filamentous stages of prothallial development. Gametophytes of *Didymochlaena truncatula* differ from these genera in having prothallial development of the *Adiantum*-type and lacking trichomes. Finally, with the exception of *Didymochlaena truncatula* we did not find important differences among the different taxa of the Dryopteridaceae.

Acknowledgments

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